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With the compliment of A.H.R. Buller

DISCOVERY OF THE FUNCTION

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(*Reprinted from* NATURE, *November* 26, 1927.)

DISCOVERY OF THE FUNCTION OF THE PYCNIA OF THE RUST FUNGI

In a letter to Nature, published July 23, 1927, I showed, on the basis of experimental evidence, that *Puccinia helianthi* is heterothallic. The results of further experiments now enable me to state definitely that *Puccinia graminis* is also heterothallic. Moreover, since my first letter was written, proof has been obtained that the pycnia (spermogonia) of the Rust Fungi are not, as many botanists have supposed, male conceptacles producing non-functional spermatia, but are active organs having a non-male function which they carry out through the agency of flies. This discovery was made as follows.

In May, 1927, I had a large number of sunflower seedlings, upon the young foliage leaves of which there were many pustules of *Puccinia helianthi*. Each pustule had originated from a single sporidium and had numerous pycnia on its upper surface, and every pycnium had excreted a drop of nectar containing pycnospores. Theoretically, as set forth in my former letter, it seemed reasonable to suppose that the mycelium, pycnia, and pycnospores of some of the pustules were (+) in sex, whereas the mycelium, pycnia, and pycnospores of other pustules were (–) in sex.

On May 17, Prof. A. H. Reginald Buller, of the University of Manitoba, was in the greenhouse of the Dominion Rust Research Laboratory inspecting the experiments in progress. A solitary fly, one of the first to appear after the winter season, had entered the greenhouse. Prof. Buller directed my attention to the fact that the fly was settling on the sunflower leaves, sipping nectar at the pycnia of one pustule and then flying off to another leaf and sipping the nectar of the pycnia of another pustule, and he at once said: "The solution of the problem of the function of the pycnium is an entomological one. Copy the action of the fly. Take (+) pycnospores to (–) pycnia and (–) pycnospores to (+) pycnia, and

it may well be that the pycnospores will germinate and bring on the diploid phase of the mycelium, evidence of which will be given by the development of æcia and æciospores on the under side of each pustule." This suggestion has been tested experimentally during the past summer and its excellence has been amply demonstrated. The experiments bearing on the function of the pycnia will now be set forth.

In two sets of experiments with *Puccinia helianthi* on sunflower leaves, pustules of monosporidial origin, each pustule having developed numerous pycnia but no æcia, were treated as follows: in 184 pustules the pycnospore-containing nectar was mixed with the help of a scalpel, the nectar of any one pustule being mixed with nectar of several other pustules; while, as a control, in 174 pustules the nectar of each pustule was stirred up with a scalpel, but not mixed with any other nectar, the scalpel being carefully sterilised before each operation.

Five days after the experiment had begun, the condition of the pustules was as follows: of the 184 mixed pustules 176 had produced æcia, 4 no æcia, and 4 had wilted and died through leaf-injury; of the 174 unmixed pustules only 20 had produced æcia, while 154 were entirely free from æcia. Under normal conditions when the nectar is neither mixed nor stirred, a certain percentage of monosporidial pustules always produces æcia, as already recorded in my first letter. The appearance of æcia in 20 of the unmixed pustules was therefore in agreement with expectation.

From the experiments just recorded it is clear that mixing the pycnospore-containing nectar leads with rapidity and considerable certainty to the development of æcia. While the pycnospores are haploid, the æciospores are diploid. We can therefore also say that mixing the pycnial nectar causes each pustule of monosporidial origin to change from the haploid to the diploid phase.

Experiments similar to those just described have been made with *Puccinia graminis* on barberry leaves. In one set of experiments the pycnial nectar of 116 monosporidial pustules was mixed; while, as a control, the pycnial nectar of each of 85 monosporidial pustules was stirred up separately but not mixed with any other nectar.

Six days after the experiment had begun, the condition of the pustules was as follows: of the 116 mixed pustules 102 had produced æcia and 14 no æcia;

whereas of the 85 unmixed pustules only 17 had produced æcia, while 68 were free from æcia.

In the experiments with *Puccinia graminis* just described we again have clear evidence of the function of the pycnia; for, when the nectar is mixed, æcia are rapidly formed in most of the pustules, whereas

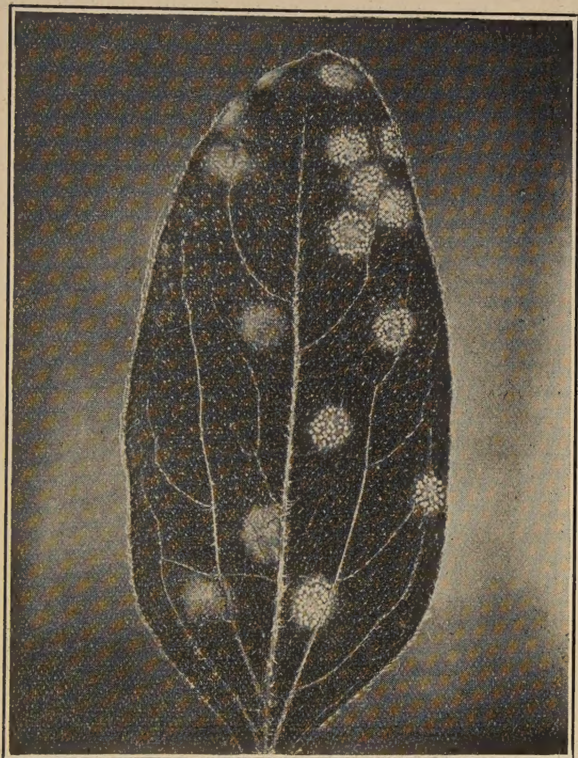

FIG. 1.—Under side of a sunflower leaf. × 1½.

when the nectar is not mixed, most of the pustules do not develop æcia. A certain percentage of unmixed pustules always produces æcia, as in *Puccinia helianthi*.

In Fig. 1 is shown the under side of a sunflower leaf. The leaf was inoculated with sporidia of *Puccinia helianthi* on July 9. Each pustule originated from a

single sporidium and was therefore unisexual. On July 29 the pycnial nectar of the ten pustules on the right side of the leaf was well mixed; while, as a control, the pycnial nectar of each of the six pustules on the left side of the leaf was stirred separately but not mixed. On Aug. 3 the leaf had the appearance shown in Fig. 1, and on Aug. 4 the photograph was taken. This experiment again clearly demonstrates

Fig. 2.—Under side of a barberry leaf. × 2.

that the pycnia are functional, in that their pycnospore-containing nectar, when transferred from one pustule to another, brings on the diploid phase as shown by the appearance of æcia within five days of the transference.

In Fig. 2 is shown the under side of a barberry leaf. The leaf was inoculated with sporidia of *Puccinia graminis* on Aug. 2. Each pustule originated from a single sporidium and was therefore unisexual. Up to Aug. 19 one pustule on the right side of the leaf

had produced æcia. On that day, the pycnial nectar of all the pustules on the right side of the leaf was well mixed; while, as a control, the pycnial nectar of each of the eight pustules on the left side of the leaf was stirred separately but not mixed. On Aug. 28 the leaf was photographed and had the appearance shown in Fig. 2. The effect of mixing the pycnial nectar is very evident: æcia appeared on the right side of the leaf where mixing had been effected, but not on the left side where mixing had been avoided.

Proof that flies mix the pycnial nectar of separate unisexual pustules and so cause the pustules to change from the haploid to the diploid phase, as shown by the appearance of æcia, was obtained with *Puccinia helianthi* as follows:

Fifteen to twenty flies were enclosed in a large screen-wire cage with about twelve pots of sunflower seedlings, on the foliage leaves of which there were 98 monosporidial pustules bearing pycnia but no æcia. As a control, flies were kept out of another large screen-wire cage which contained fifteen pots of sunflower seedlings, on the foliage leaves of which there were 159 similar pustules.

Eight days after the beginning of the experiment 96 of the 98 pustules to which flies had had access had produced æcia and only 2 pustules no æcia, whereas only 5 of the 159 pustules to which flies had not had access had produced æcia.

It was found that in *Puccinia helianthi*, and also in *P. graminis*, nectar which had been heated to 70° C. to kill the pycnospores is not effective in inducing the production of æcia when mixed with the nectar of other pycnia on the living leaf. This indicates that it is the pycnospores which are the effective agents in inducing the formation of aecia, and not the nectar.

In a series of experiments with *Puccinia helianthi*, and in another series with *P. graminis*, the pycnial nectar of one monosporidial pustule was removed in a capillary tube and divided into several drops, and then the drops were applied singly to the pycnia of as many pustules as there were drops. In response to this treatment some of the pustules produced æcia and others did not, thus indicating that the pycnospores are of two kinds, (+) and (–). The full details of these experiments will be recorded elsewhere.

It appears that, under natural conditions, there are three ways in which pustules of monosporidial origin may change from the haploid to the diploid condition: (1) by a (+) sporidium and a (–) sporidium settling on a leaf close together, so that they form pustules which

coalesce in such a way that the (+) mycelium and the (–) mycelium come into contact directly ; (2) by means of flies which carry (+) pycnospores from one isolated pustule to the (–) pycnia of another isolated pustule or, conversely, (–) pycnospores of one isolated pustule to the (+) pycnia of another isolated pustule ; and (3) spontaneously. The cause of the spontaneous change of a certain number of the pustules of *Puccinia helianthi* and of *Puccinia graminis* from the haploid to the diploid condition is at present unknown, but the phenomenon finds its parallel among the Hymenomycetes in *Coprinus radians* investigated by Vandendries and in *C. Rostrupianus* investigated by D. E. Newton.

The pycnia attract flies and reward them for their visits in very much the same way as do flowers or the Stinkhorn Fungus. They occur chiefly on the upper side of the leaves, where they are readily accessible to insects ; they are usually yellow or red in colour, by which means—and perhaps also by the refraction and reflection of light in the drops of nectar—they are made conspicuous ; in some species, *e.g. Puccinia suaveolens*, and possibly in many, they emit an attractive odour ; while, finally, the nectar contains sugar, and on this account is sipped by flies with avidity.

It has long been remarked that, in those rust fungi which possess them, the pycnia are the first spore-producing organs to appear. Since they play such an important part in changing the haplophase into the diplophase and in inducing the formation of æcia, their appearance on the mycelium before the æcia can now be readily understood. Pycnia precede æcia, because by pycnial action æcia are formed.

The crossing of two physiological forms of *Puccinia graminis*, etc., might be effected in the following relatively simple manner : obtain monosporidial pustules of both strains and then mix the pycnial nectar of a (+) pustule of one strain with the nectar of a (–) pustule of the other strain, or, conversely, mix the nectar of a (–) pustule of one strain with the nectar of a (+) pustule of the other strain.

In my previous letter to Nature I stated that, in *Puccinia helianthi*, the æciospores which had appeared in at least some of the æcia of pustules of monosporidial origin are uninucleate. Further cytological experience has convinced me that the apparent uninucleate condition of these æciospores was due to an artefact. The young æciospores near the spore-bed of every æcium of monosporidial origin that I have

investigated more recently have all proved to be binucleate.

In conclusion, I desire to thank Prof. Buller for assisting me with valuable suggestions and helpful criticism.

J. H. Craigie.

The Dominion Rust Research Laboratory,
Winnipeg, Oct. 13.

Printed in Great Britain by R. & R. CLARK, LIMITED, *Edinburgh.*